ON THE CYTOLOGY OF SYNCHYTRIUM.

III. The Role of the Centrosome in the Reconstruction of the Nucleus.*

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INTRODUCTORY. The first paper of this series on the cytology of Synchytrium was published by Dr. and Mrs. F. L. Stevens, who took up the study fresh from their remarkable work on the cytology of the Oomycetes, hoping to clear up the very doubtful relationships of the Chytridiales which as they say "have offered an open field for speculation heretofore and have baffled definite judgments as to their relationships." They soon saw, however, that they had a very large problem on their hands because of the many anomalous structures encountered which were very difficult to reconcile with the cytology of the higher organisms. They therefore held the "discussion of these structures for a separate paper, the present one being limited to a series of stages which clearly pertain to true mitosis of the primary nucleus." Other pressing work kept Professor Stevens away from Synchytrium, however, and he decided to turn over his material to the present writer. Some of the structures he saw he has described in a second article ('07). With these suggestions as to the interesting features in the cytology of the group, the writer has been fortunate indeed in having furnished him ready to work with all of Stevens' material, alcoholic, paraffine cakes and slides, as well as notes of observation on the slides. He is therefore under very considerable obligations to Professor Stevens, who, forced to forego the pleasure of working out these structures personally, has yet followed the study with a keen interest which quite justifies the consideration of this as a continuation of his former work on the same object.

Before I took up the material, however, Mr. Lon A. Hawkins, formerly fellow in Botany at the Ohio State University, undertook the investigation but was compelled to drop it on assuming work for the government. It was his preparations that were used in this present work and the writer is very considerably indebted to Mr. Hawkins also for his slides. They are beautiful preparations cut from 2 microns thick and stained in Iron Haematoxylon. Stevens's preparations were stained with the triple stain.

For the rest of the work, Dr. Stevens and I propose to take up in detail, step by step the peculiar cytological structures in this interesting group either separately or jointly as circumstances

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may favor, with the hope of correlating the cytology of Synchytrium with that of other plants and animals, in a way which may throw some light on some general problems of cell organization and finally to arrive at some conclusions regarding the relationships of the Chytridiales.

OBSERVATIONS.*

After the division of the primary nucleus of *Synchytrium decipiens* which species alone was used in the present investigation, the secondary nuclei divide rapidly without the formation of cell walls till segmentation takes place when there are usually 500–800 nuclei in the cyst. Kusano reports that all these mitoses are similar in *S. puerariae* and such seems to be the case in *S. decipiens*, the only change being in the continual diminution in the size of the nucleus. But while it is believed that all of the mitoses are similar it must be remarked that all of the observations here presented, were made on cysts about midway between the primary cell and the segmented sorus in respect to the number of nuclei, i. e., from cysts with 100–300 nuclei. Whether this has any significance or not we do not at present know.

The spindles of Synchytrium like those of fungi generally, arise within the nucleus and reach metaphase before the nuclear membrane is dissolved from around them. As Stevens found both in the primary mitosis and in the succeeding ones, no centrosomes are demonstrable at the poles. The figure made by the separated chromosomes at each pole in anaphase (fig. 1) resembles greatly that of the same stage in the primary division, compare Stevens's (03) fig. 13 with my fig. 1, thus strengthening the statement that all the mitoses are similar. There appear to be four chromosomes as Stevens supposed though it is sometimes difficult to see more than three (cf. figs. 1, 2, 8. In figure 1 there are four chromosomes at the pole where only three can be seen, one being directly beneath the one shown nearest the nucleolus.)

By the beginning of the telophase the daughter nuclei are separated by an unusually great distance from each other. In the mitoses from which the present figures were drawn they are about 20 microns apart (see figs. 1 and 12.) Such a condition as is shown in figure 5 where the daughter nuclei lie close together is quite unusual. It is readily seen that in thin sections the

* Since the observations herein recorded were made but before they were embodied in their present form Kusano (07) has published a preliminary paper on *Synchytrium puerariae* in which he announces the same relation of the centrosomes to the nuclear membrane as is herein described. Though he gives five figures they are hardly sufficient to demonstrate his point and his fuller paper is to be much desired inasmuch as the action of the centrosomes of Synchytrium are so unusual that confirmation of the results by independent workers will undoubtedly be welcomed by the cytological fraternity.

chances of securing favorable sections of both the daughter nuclei are rather remote. In the present case with sections 2 microns thick, the chance is only about one in twenty-five, neglecting the thickness of the nuclei though they are of about the same thickness as the sections which reduces the chances very materially. It is evident, then, that it is not easy to get a full series of nuclei in which both members of the pair show. Most of the drawings therefore delineate only one nucleus.

While centrosomes are not demonstrable on the spindles at metaphase or early anaphase (fig. 1 cf. Stevens 07 figs. 18–21), in what is interpreted as telophase (fig. 2) there are found enormous asters. The manner of their appearance has not yet been made out. Though usually located near the former axis of the spindle they are by no means accurately placed at its poles as can be seen by consulting almost any of the figures. In structure also it may be seen that they vary greatly. Sometimes there is a single deeply staining granule (centrosome proper) at the centre of the radiations (figs. 6, 7, 8, 11); sometimes the deep staining taken at the centre seems to be due simply to the convergence of radiations (fig. 5). In only one case and that not very distinct, was there seen anything approaching a clear centrosphere around the central granule. But very often there is more than one granule. The different granules may be located at the focus of a single aster so as to look like a dividing centrosome (fig. 2). They may be scattered about the focus without any very definite relation to the rays(fig. 3). More often the different granules are the centres of separate asters so that there appear two centrosomes (fig. 2, 4). Rarely there may be more than two distinct centrosomes; figure 9 shows a case where there were three connected by heavy fibrous bands of kinoplasm, while each has its own aster. Figure 10 shows an anomalous condition where the centrosomes are located at nearly opposite poles of the nucleus while their rays meet so as to extraordinarily resemble the amphiaster common in animal mitosis. Judged by itself this nucleus would seem to be in the prophase of division for in addition to the amphiaster the chromatin is arranged similarly to the spirem of the prophase. Such an interpretation seems, however, entirely inadmissible, since the chromatin in prophase does not, so far as is now known, assume such a spirem and the spindle, being intranuclear, has no relation to any such amphiaster. This condition was seen only in the single nucleus located in a cyst where all the other asters conform to the usual type. Fig. 7, however, shows a condition interesting in comparison; though this may be simply a case where the second nucleus of a pair was cut out of the section, leaving a part of its aster. Here, in addition to the conspicuous aster at one pole of the nucleus, is another on the opposite side which though faint and lacking a very definite

granule is still clearly visible showing in addition to a few short rays stretching away from the nucleus others connecting with the nuclear membrane.

The various irregularities in the centrosomes are to be compared in the judgment of the writer, to those present in the formation of centrosomes de novo, under more or less abnormal chemical stimulation, in animals. In many of these cases there are formed a multitude of small asters two of which grow large and form the amphiaster. Because of the difficulty of determining the sequence of events, having no other indications of the relative ages beside the condition of the asters themselves, it cannot be asserted that the different asters coalesce or that one of them gains the mastery while the other disintegrates. Nevertheless it is to be noted that without exception those asters which are interpreted as the end stages were, so far as seen, uniformly single (figs. 12–14).

The activities of the centrosomes and of the chromatin in the reconstruction of the nucleus are apparently independent of each other to a considerable degree so that it seems necessary to consider them separately. In the telophase the four chromosomes lie loosely in the cytoplasm making a figure not unlike the typical daughter star. They have a manifest tendency to converge to the centrosome which is more clearly shown in cases where there are two asters, when part of the chromosomes may follow the rays of each. At the distal ends of the chromosomes, with respect ot the aster, there soon appears, in connection with one or more of them (fig. 2) a thickening which enlarges at the expense of the chromosomes till it becomes the karyosome (nucleolus) of the resting nucleus (fig. 2). The transfer of the chromatin to the karyosome consists apparently, not so much in the direct absorption of the chromosomes as in the gradual removal of the chromatin from the linin matrix so that in many cases the ends of the chromosomes farthest away from the karyosome become vacuolate, as it were, and lose their staining reaction giving a gradual transition from one condition to the other thereby showing apparently that the chromatin migrates granule by granule from the linin matrix (fig. 2.) Those chromosomes which do not directly connect with the growing karyosome form linin bridges across to it, by which the chromatin may be transferred.

In many cases the nuclear membrane is formed before this process is complete and there results a spirem which closely resembles that usually found in the prophase of dividing cells. (figs. 7–11). In such spirems all resemblance to the original chromosomes may be lost and a loose, few-meshed network formed. In as much as the conduct of the chromatin and of the asters are independent, some doubt is thrown on the exact sequence of the transformations of the nucleus as well as of the cen-

trosomes. This is particularly true of the stage represented by fig. 14, where the chromatin is in spirem in every one of the nuclei in the three cysts observed although the aster has apparently run its course. This suggests that we have not reached a full understanding of the phenomena as yet. Nevertheless in any case it seems certain that the resting nucleus, with its chromatin all or nearly all concentrated in the single globular karyosome (figs. 12–13) must be connected with the chromosomes of anaphase by a series of stages not far divergent from these here described.

The cycle of astral activity which is the main interest of the present paper may now be taken up. When at their maximum size the rays are relatively few, long, and so thick as to be clearly visible under a low magnification. They are not always straight (fig. 7) and do not always center exactly in the focus of the aster (fig. 12). Quite often they have thickenings or granules along their length (figs. 2, 4, 5, 12). These are apt to be located at the intersections of the rays with the strands of the cytoreticulum (fig. 5). At later stages the central deep staining granule gives place to a larger diffuse granular area in which there may be still some deeper staining granules but they are more minute than those which preceeded them. The rays at the same time become finer and more numerous till they resemble the spindle fibres in ordinary mitosis. The granular area then appears to enlarge while the rays disappear and finally the centrosome seems to become simply a densely granular mass of cytoplasm which does not stain more deeply than the general reticulum (fig. 14). In this stage the centrosome resembles greatly the so-called attraction spheres of some animal cells, e. g., some stages of Ascaris. This mass is then dissipated into the general cytoplasm by imperceptable stages thus leaving the nucleus without centrosomes as it began. As before indicated the condition of the nucleus at this time throws some doubt upon this sequence so that the history of the centrosome, like that of the nucleus, may be subject to some revision. But it appears sure that the centrosome arises de novo out of the cytoplasm and disintegrates into cytoplasm again whether the sequence of events be exactly that given or not.

The remarkable feature of the aster, however, is the relation of the rays of the centrosome to the reconstruction of the nucleus. At an early stage a vacuole appears around the chromosomes. This is at first quite without a membrane (fig. 2) but very soon those astral rays which are nearby come to form a cone enclosing it (figs. 3, 4). Soon the ends of the rays bend around the vacuole and enclose it *forming the nuclear membrane* (figs. 5, 6, 7). These membrane-forming rays may be observed to taper greatly from the center toward the curved ends (figs. 4, 7). At their thicker ends they are very heavy indeed and stain deeply so that they

could not be accurately represented by anything less than the heavy lines in the drawing. Since the rays are cylindrical rods rather than plates the membrane is not a first any membrane at all but a cage around the vacuole. The method by which the interstices between the bars are filled up could not be followed satisfactorily. But it may be that the substance of the rays gradually spreads out around the vacuole till the membrane is completely formed. This process may be seen on the side of the nucleus towards the centrosome in those cases where the apex of the cone of rays is very acute. Here the rounded surface of the vacuole may be seen to acquire a membrane like that of the rest of the nucleus so gradually that it is often difficult to tell whether the membrane is present or absent (fig. 11). In these cases the cap of rays persists for a considerable time but gradually fades when the membrane is complete (figs. 10,11).

This method of the formation of the nuclear membrane by the rays of the centrosome has been observed by the writer in very many cases; to the five figures given to illustrate the stages of the process could be added many more if it were deemed necessary.

Aside from the peculiar method of its formation, the nuclear membrane of Synchytrium is a remarkable structure. In the primary nucleus it reaches a relatively enormous thickness (See Stevens '03, fig. 5) and it is so stiff that it is often broken and carried away by the knife. In the succeeding mitoses it is not only thickened but sometimes presents some very peculiar aspects which we shall hope to deal with in a later paper. One feature may be touched upon here.

Those rays which form the membrane, like the others, frequently have granules strung along them. In other rays the granules are centrally placed on the ray but in these they are nearly always found on the *inside* of the nuclear cavity (figs. 4, 7, 8). In older stages they may be found either within the nucleus, in the wall, or lying against its outer edge (fig. 12). Perhaps, correlated with these granules are others frequently seen loose in the cytoplasm, and surrounded each by a vacuole of its own (fig. 11). But consideration of these would carry us too far afield. We can not do more at this time than to suggest the possible analogy between the formation of these granules and the derivation of the microsomes of the cytoplasm from the nucleus as described by Lillie and others.

SUMMARY. The exact history of the structures touched incidentally, the asters and the chromatin content of the nucleus, is somewhat provisional, but the point of the present paper is the demonstration, confirming and amplifying Kusano's announcement that the rays of the centrosome enclose the vacuole surrounding the naked chromosomes, and form a very heavy deeply staining membrane around it, the nuclear membrane.

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EXPLANATION OP PLATES XIX AND XX.

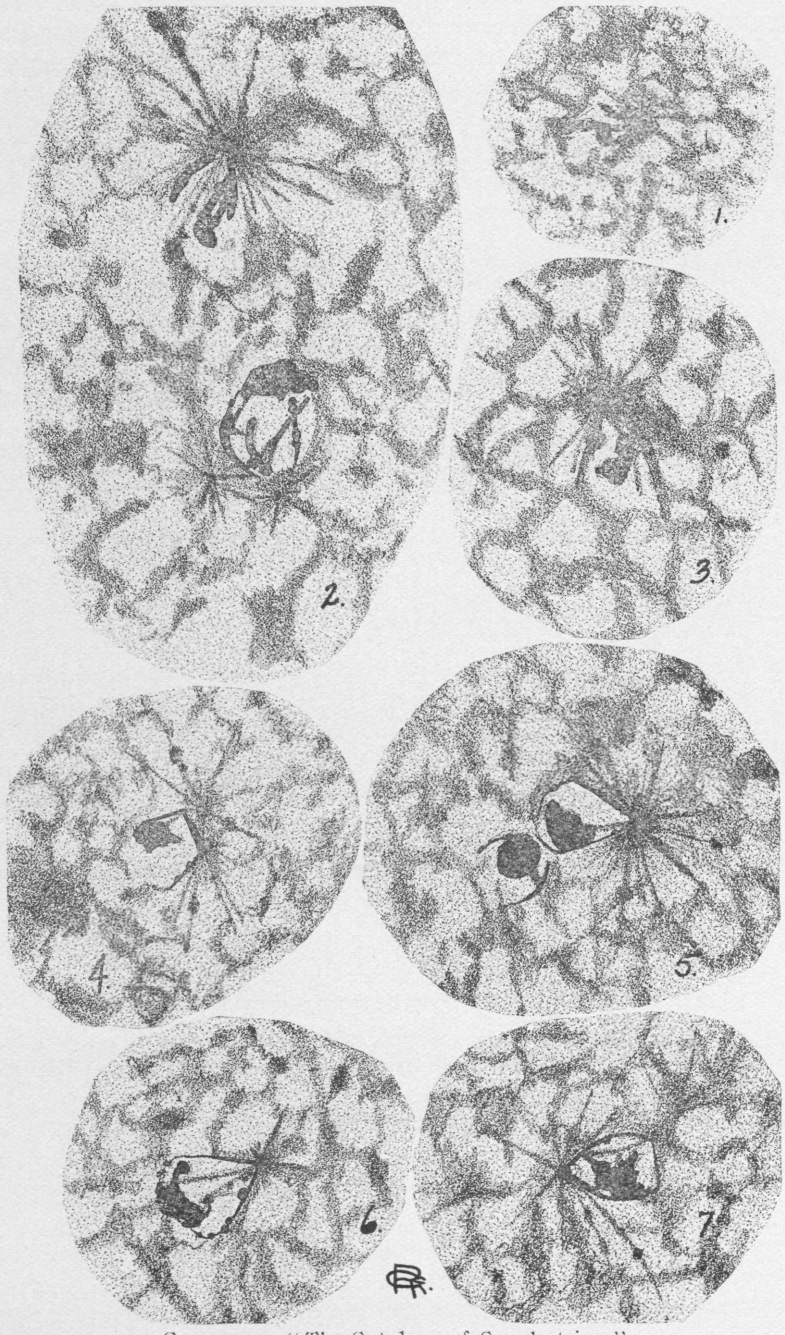
The figures were made with a Spencer 1.5 mm. achromatic, oil immersion objective and a Zeis compensating ocular 12 giving at the table a measured magnification of approximnately 4000 diameters. They were reduced to 2–3 of their original size exactly canceling the enlargement due to the camera and rendering the figures the same size as they were seen in the field of the microscope, namely enlarged 2670 times. All are camera drawings.

Fig. 1. Early anaphase; a blob of chromatin at one pole, at the other 4 chromosomes, one covering another.

Fig. 2. Telophase considerably later than fig. 1.Showing the process of transformation of the chromosomes and also the beginning of the nuclear vacuole which is without a membrane.

Fig. 3. Nucleus in wich the rays are beginning to be associated with the edges of the nuclear vacuole.

Fig. 4. Nucleus with two asters the rays of each of which are beginning to form the nuclear membrane.



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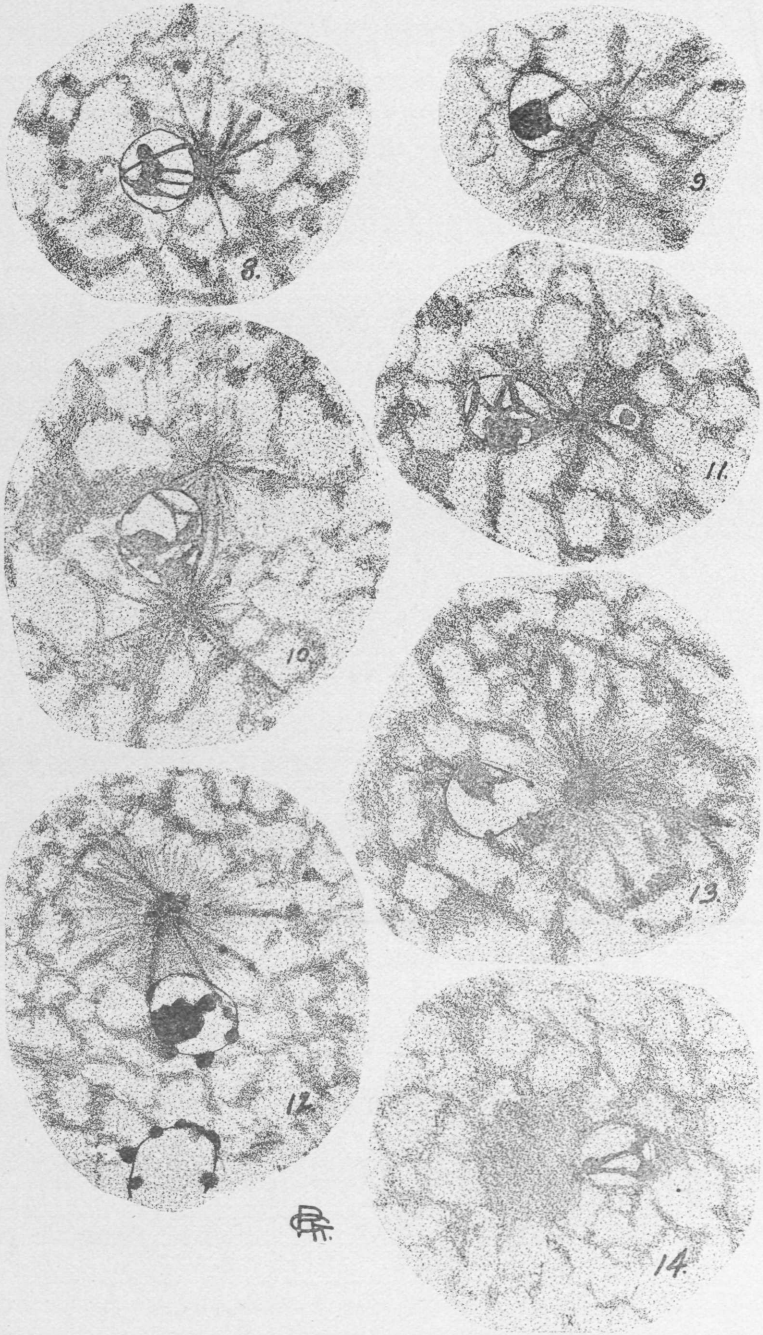

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Fig. 5. A pair of daughter nuclei remaining unusually close together (one cut in sectioning); rays of the centrosome prominent and granular, bending around the nuclear vacuole on the side opposite the centrosome, but not yet met to complete the membrane.

Fig. 6. A cone of tapering astral rays enfolding the nuclear cavity and bending around beyond it; prominent granules on the nuclear membrane.

Fig. 7. Muclear membrane almost complete by the meeting of the rays.

Fig. 8. Nucleus showing a banded condition similar to a spirem; the bands probably derived from the four chromosomes.

Fig. 9. A nucleus with three distinct asters connected by heavy radiations.

Fig. 10. A nucleus in a spirem-like condition with two centrosomes forming, with their radiations, a figure like an amphiaster.

Fig. 11. A nucleus showing the gradual genesis of the nuclear wall on the side next the centrosome; also a large deep-staining granule surrounded by a vacuole.

Fig. 12. An aster with a large rather diffuse centre and numerous very fine rays; nuclear membrane complete but still associated with the rays. On the membrane of both this and the portion of the sister nucleus are conspicuous granules lying in different positions with respect to the nuclear wall.

Fig. 13. Centrosome more diffuse than in fig. 12; nuclear membrane disturbed by knife.

Fig. 14. Nucleus in spirem sage with a large mass of dense cytoplasm at one side which is interpreted as the end stage of the centrosome.